

§ 113.301

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each serial or first subserial of live virus vaccine recommended for animals other than poultry shall be tested for safety in at least one species for which the vaccine is intended using methods prescribed in §§ 113.39, 113.40, 113.41, 113.44, and 113.45 or in a filed Outline of Production. The mouse safety test prescribed in § 113.33(a) shall also be conducted unless the virus or agent in the vaccine is inherently lethal for mice.

(c) *Virus identity test.* At least one of the virus identity tests provided in this paragraph or a suitable identity test prescribed in the filed Outline of Production shall be conducted on the Master Seed Virus and final container samples from each serial or first subserial of biological product.

(1) *Fluorescent antibody test.* The fluorescent antibody test shall be conducted using virus inoculated cells and uninoculated control cells. Cells shall be stained with fluorochrome conjugated specific antiserum. Fluorescence typical of the virus concerned shall be demonstrated in the inoculated cells. The control cells shall remain free of such fluorescence.

(2) *Serum neutralization test.* The serum neutralization test shall be conducted using the constant serum-decreasing virus method with specific antiserum. For positive identification, at least 100 ID₅₀ of vaccine virus shall be neutralized by the antiserum.

(d) *Cell Culture Requirements.* If cell cultures are used in the preparation of Master Seed Virus or of the vaccine, primary cells shall meet the requirements prescribed in § 113.51, cell lines shall meet the requirements prescribed in § 113.52, and ingredients of animal origin shall meet the applicable requirements in § 113.53.

(e) *Moisture content.* The maximum moisture content in desiccated vaccines shall be stated in the filed Outline of Production.

[39 FR 27430, July 29, 1974, as amended at 43 FR 49528, Oct. 24, 1978; 50 FR 1042, Jan. 9, 1985; 54 FR 19352, May 5, 1989. Redesignated at 55 FR 35562, Aug. 31, 1990; 60 FR 24549, May 9, 1995]

§ 113.301 Ovine Ecthyma Vaccine.

Ovine Ecthyma Vaccine shall be prepared from tissue culture fluids or virus-bearing tissues obtained from

sheep that have developed ovine ecthyma following inoculation with virulent ovine ecthyma virus. Ovine Ecthyma Vaccine is exempt from the requirements prescribed in §§ 113.27 and 113.300(a), (b), and (c). Each serial shall meet the moisture requirements in § 113.300(e) and the special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests.* (1) Bulk or final container samples of completed product from each serial shall be tested for safety as prescribed in § 113.38.

(2) The prechallenge period of the potency test shall constitute a safety test. If unfavorable reactions attributable to the vaccine occur in either of the vaccinates during the observation period, the serial is unsatisfactory.

(b) *Potency test.* Final container samples of completed product from each serial and each subserial shall be tested for potency using susceptible lambs. The vaccine shall be prepared as recommended for use on the label.

(1) Each of two lambs (vaccinates) shall be vaccinated by application of the vaccine to a scarified area on the medial surface of the thigh and observed each day for 14 days.

(2) The immunity of the two vaccinates and one or more unvaccinated lambs (controls) shall be challenged in the same manner as for vaccination, using the opposite thigh.

(3) If typical signs of ovine ecthyma, such as hyperemia, vesicles, and pustules do not develop on the controls during the first 2 weeks following challenge and persist for approximately 30 days, the test is inconclusive and may be repeated.

(4) If the vaccinates do not show a typical immune reaction, the serial is unsatisfactory: *Provided*, That, an initial active reaction with hyperemia which resolves progressively and disappears within 2 weeks, may be characterized as a typical immune reaction.

[39 FR 27430, July 29, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.302 Distemper Vaccine—Mink.

Distemper Vaccine—Mink shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus

which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in §113.300 and the requirements prescribed in this section.

(b) The lot of Master Seed Virus shall be tested for extraneous viruses as follows:

(1) To detect virulent canine distemper virus, each of two distemper susceptible mink or ferrets shall be inoculated with 1 ml of the Master Seed Virus and observed each day for 21 days. If undesirable reactions occur in either test animal, the lot of Master Seed Virus is unsatisfactory.

(2) Master Seed Virus propagated in chicken embryos shall be tested for pathogens by the chicken embryo test prescribed in §113.37 except lesions typical of distemper virus may be disregarded. If found unsatisfactory, the Master Seed Virus shall not be used.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) At least 25 distemper susceptible mink shall be used as test animals. Blood samples shall be drawn from these animals and individual serum samples tested. The mink shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID₅₀ of canine distemper virus. Other means of insuring susceptibility may be used if prior approval from Animal and Plant Health Inspection Service is received.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. At least 20 mink shall be vaccinated with a predetermined quantity of vaccine virus and at least 5 additional mink shall be held as unvaccinated controls. To confirm the dosage calculations,

five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least twenty-one days post-injection, the immunity of each of the vaccinates and the controls shall be challenged with the same size dose of virulent distemper virus and observed each day for 21 days.

(i) If at least 80 percent of the controls do not die or show severe signs of distemper, the test is inconclusive and may be repeated.

(ii) If at least 19 of 20, 27 of 30, or 36 of 40 of the vaccinates do not survive without showing clinical signs of distemper during the observation period, the Master Seed Virus is unsatisfactory.

(4) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates and five controls need to be used in the retest; *Provided*, That five of the five vaccinates and at least four of the controls shall meet the criteria prescribed in paragraph (c)(3) of this section.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed Virus shall be authorized by Animal and Plant Health Inspection Service.

(d) Test requirements for release: Each serial and subserial shall meet the general requirements prescribed in §113.300 and the requirements in this paragraph. Final container samples of completed product shall be tested. Any serial or subserial found unsatisfactory by a prescribed test shall not be released.

(1) *Mink safety test.* Each of 2 mink shall be vaccinated with the equivalent of 10 doses of vaccine rehydrated with sterile diluent and administered in the manner recommended on the label. The mink shall be observed each day for 21 days. If unfavorable reactions attributable to the product occur in either of the mink during the observation period, the serial or subserial is unsatisfactory. If unfavorable reactions which are not attributable to the product occur, the test shall be declared inconclusive and may be repeated; *Provided*, That if the test is not repeated, the serial or subserial shall be declared unsatisfactory.

(2) *Potency Test.* An in vitro potency test shall be conducted. To be eligible for release, each serial and subserial shall have a virus titer sufficiently greater than the titer of vaccine virus used in the immunogenicity test prescribed in paragraph (c) of this section to assure that, when tested at any time within the expiration period, each serial and subserial shall have a virus titer $10^{0.7}$ greater than that used in such immunogenicity test when tested by the method used in paragraph (c)(2) of this section.

[40 FR 53000, Nov. 14, 1975, as amended at 48 FR 33471, July 22, 1983. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66784, 66786, Dec. 26, 1991]

§ 113.303 Bluetongue Vaccine.

Bluetongue Vaccine shall be prepared from virus-bearing cell culture fluids. Only Master Seed which has been established as pure, safe, and immunogenic shall be used for preparing the seeds for vaccine production. All serials of vaccine shall be prepared from the first through the tenth passage from the Master Seed.

(a) The Master Seed shall meet the applicable general requirements prescribed in § 113.300 and the requirements in this section.

(b) Each lot of Master Seed shall be tested for transmissibility and reversion to virulence in sheep using a method acceptable to Animal and Plant Health Inspection Service. If reversion to virulence is demonstrated, the Master Seed is unsatisfactory.

(c) Each lot of Master Seed used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed shall be established as follows:

(1) Twenty-five lambs, susceptible to the bluetongue virus serotype contained in the vaccine, shall be used as test animals (20 vaccinates and 5 controls). Blood samples shall be drawn from these animals and individual sera tested. A lamb shall be considered susceptible if there is no neutralization at a 1:2 final serum dilution in a constant virus varying serum neutralization test with 60 to 300 TCID₅₀ of bluetongue virus or another method acceptable to Animal and Plant Health Inspection Service.

(2) A geometric mean titer of the vaccine produced from the highest passage from the Master Seed shall be established before the immunogenicity test is conducted. The 20 lambs to be used as vaccinates shall be administered a predetermined quantity of vaccine virus by the method recommended on the label. To confirm the virus dosage administered, five replicate virus titrations shall be conducted on a sample of the vaccine used.

(3) At least once during the period of 14 to 18 days postvaccination, individual serum samples shall be collected from each of the vaccinates and tested for virus neutralizing antibody using the 60 to 300 TCID₅₀ of bluetongue virus.

(4) Twenty-one to twenty-eight days postvaccination the vaccinates and the controls shall each be challenged with virulent bluetongue virus and observed for 14 days. The rectal temperature of each animal shall be taken and recorded for 17 consecutive days beginning 3 days prechallenge. The presence or absence of lesions or other clinical signs of bluetongue noted and recorded on each of 14 consecutive days postchallenge.

(i) If at least four of the five controls do not show clinical signs of bluetongue and a temperature rise of 3 ° F or higher over the prechallenge mean temperature, the test shall be considered inconclusive and may be repeated.

(ii) If at least 19 of the 20 vaccinates tested as prescribed in paragraph (c)(3) of this section do not have bluetongue neutralizing antibody titers of 1:4 final serum dilution or higher, or if more than one of the vaccinates shows a temperature rise of 3 ° F or higher than its prechallenge mean temperature for 2 or more days, or if more than one of the vaccinates exhibits clinical signs of bluetongue, the Master Seed is unsatisfactory.

(5) An Outline of Production change shall be made before authority for use of a new lot of Master Seed shall be granted by Animal and Plant Health Inspection Service.

(6) The Master Seed Virus shall be retested for immunogenicity in 3 years unless use of the lot previously tested is discontinued. Only five vaccinates